REMARKS

Claims 1, 31, and 40 were previously cancelled from the case. Claims 7, 32-39, and 42-45 are cancelled herein. Such cancellation is without prejudice on the merits to further prosecution of the subject matter of these claims in one or more continuing applications.

Claims 46-48 are newly added herein. Support for these claims can be found in the specification at page 15, 1st, 2nd, and 3rd full paragraphs; at page 17, 2nd and 3rd full paragraphs; and at page 58, 3rd full paragraph.

Claims 2-6, 8-30, and 41 are amended herein. Support for the language in Claim 2 stating that genomic DNA is isolated "without an intervening step of culturing cells" from the source can be found in the specification at page 13, lines 12-13. Support for cloning genomic DNA wherein at least some of the cloned DNA has a length of at least 50, 75, or 100 kb is presented in the specification at page 5, lines 12-13. Support for the recitation that the cloned DNA has an "average" length of at least 50, 75, or 100 kb is presented in the specification at page 17, 2nd full paragraph.

No new matter is added.

Claims 2-6, 8-30, 41, 46, and 47 remain in the application. Favorable reconsideration is requested.

Change of Correspondence Address:

A Revocation of Power of Attorney; Granting of New Power of Attorney; Change of Correspondence Address accompanies this paper. Please forward all future correspondence in this application to:

Intellectual Property Department
DeWitt Ross & Stevens S.C
8000 Excelsior Drive, Suite 401
Madison, WI 53717-1914

A Statement Under 3.73(b) also accompanies this paper.

Objections to the Claims:

The objections to Claims 7, and 42-45 have been rendered moot by cancellation of the claims.

The objections to Claims 6, 12, 14, 25, and 26 have been addressed by appropriate amendment, in accordance with the Examiner's recommendations. The spelling of the words "microorganism," "anaerobes," and "Agrobacterium" has been corrected throughout the claim set.

Claims 10 and 11 have been amended so that they parallel the language of Claims 14 and 15, in accordance with the Examiner's recommendation.

Rejection of Claims 2 and 33 Under 35 USC §112, First Paragraph (Written Description):

As applied to Claim 33, this rejection has been rendered moot by cancellation of the claim.

As applied to Claim 2, this rejection is believed to have been overcome by appropriate amendment to the claim.

Applicants' undersigned counsel notes that while the Office provided rather extensive comments with regard to now-cancelled Claim 33, the Office did not explicitly articulate its fundamental concern with regard to the previous language of Claim 2.

In any event, Claim 2 as amended is directed to a method for "detecting" a compound produced by a biosynthetic pathway. As positively recited in Claim 2, genomic DNA is isolated from a source containing uncultivated microorganisms, in the absence of any culturing of the cells. The isolated genomic DNA is then inserted into a vector and cloned into a host cells to yield a library of cells containing the cloned genomic DNA. The inserts in at least some of the clones must be at least 50 kb long. The hosts are then cultures under conditions wherein an open reading frame sequence of the cloned genomic DNA is expressed. A compound produced by the host cells as a result of expression of the open reading frame sequence is then detected by various means. See Claims 25-30.

As noted at page 3, lines 9-10 of the specification, the compound produced by the library of transformed host cells is detected "relative to host cells lacking the [inserted] genomic DNA." Thus, for example, spectrometric (Claim 27) or chromatographic (Claim 28) detection assays are run on the non-transformed host cells and on the transformed host cells. New compounds produced by the transformed host cells (as a

result of the transformation) are detected by comparing the spectrometric and/or chromatographic results generated by the non-transformed host cells to the corresponding results generated by the transformed host cells. A very extensive discussion of suitable detection techniques that can be used in the present invention is provided in the specification at page 29, last paragraph, to page 39, first paragraph.

Note that as amended, Claim 2 does not require that the chemical structure or "identity" of the compound be discovered or known. The method of Claim 2 is positively directed to a method for "detecting" a new compound that is produced as a result of transforming the host cell to contain the isolated genomic DNA of step (i). In other words, the utility of the method is in cloning a biosynthetic pathway from a uncultivated (and likely uncultivatable) collection of microorganisms and recapitulating that biosynthetic pathway in a host library that can be cultivated, and then "detecting" one or more compounds produced as a result of the cloned biosynthetic pathway. The information generated by the claimed method reveals valuable biochemical information about the uncultivated microorganisms present in the source material.

In terms of written description, then, the specification as filed contains an extensive description of how to isolate the required DNA, how to package that DNA into a replicable vector, and how to insert the vector into a host. The specification as filed also includes an extensive discussion (spanning pages 29 to 39) on how to analyze and compare the transformed cells to the non-transformed cells in order "to detect" new compounds produced by the transformed host cells.

Applicants thus submit that the rejection of Claim 2 under §112, first paragraph (written description) has been overcome. Withdrawal of the rejection is respectfully requested.

Rejection of Claims 2-30, 32, and 33 Under 35 USC §112, First Paragraph (Enablement):

As applied to Claim 32 and 33, this rejection has been rendered moot by cancellation of the claims.

As applied to Claims 2-30, this rejection is respectfully traversed because one does not need any prior knowledge of a compound or the nature of that compound in

order to detect its presence. (Claims 2-30 **do not** require that the chemical structure of the compound be identified, nor do these claims require that the detected compound be isolated.)

As a very famous example of the above-noted proposition, Pierre and Marie Curie were able **to detect** radium years before they were able to isolate a sufficient quantity of radium to characterize it as a new element. See Exhibit A, attached hereto, which is an excerpt of Chapter 5 of the book "Pierre Curie," authored by Marie Curie (translated from the original French by Charlotte and Vernon Kellogg, copyright 1923, Macmillan Co., New York, NY). Exhibit A was obtained on-line from "www.lateralscience.co.uk." A complete version of book can also be obtained on-line from The Electronic Text Center of the University of Virginia Library at:

http://etext.lib.virginia.edu/toc/modeng/public/CurPier.html.

In particular, see the highlighted passages at pages 3 and 7 of Exhibit A: The Curies announced the **detection** of two new radioactive species, designated polonium and radium, in July and December of 1898, respectively. However, it took them another two years of work to isolate and to characterize these two elements. Thus, without knowing a thing about the chemistry, the structure, the reactivity, the molecular weight, etc. of either polonium or radium, the Curies still were able to detect the presence of these two radioactive elements by extrapolation from what was already known, namely, the natural radioactivity of uranium and thorium. Because the known radioactivity of uranium and thorium could not account for the large amount of radioactivity emitted by the ore pitchblende, the Curies where able to **detect** the presence of additional entities that were responsible for the observed radioactivity, without knowing anything whatsoever about the structure or chemical properties of these new entities.

The same holds equally true of the present method as recited in Claims 2-30. One need only to compare what is known and thoroughly characterized—*i.e.*, the qualities of the untransformed host cell—and compare it (using any number of well-known means) to the qualities of the transformed host cells. As noted in the prior section, several well-known means for detecting chemical species are discussed in the specification at page 29 to 39. Comparing the transformed host cells to the non-transformed host cells by various means is explicitly noted in the specification at page 3, lines 9 and 10: "[detect] a

compound produced by the host cells, *e.g.*, relative to host cells lacking the genomic DNA."

Further still, Applicants explicitly traverse the statement spanning the bottom of page 7 to the top of page 8 that "One of ordinary skill... would have to envision that characteristics **of all** the possible classes of novel compounds that would be produced...." (Emphasis added.) Applicants respectfully submit that the Office is reading into the claims a limitation that does not appear in the claims. The claims **do not** require that the method reveal **all** of the compounds produced as a result of the transformation. The claims require only that "a compound," (that is, any compound) produced as a result of the transformation, be detected. Thus, for example, it is an exceedingly simple proposition to take a sample of culture medium from untransformed cells and a sample of culture medium from transformed cells, subject the samples to any number of different chromatographic separation protocols, and then compare the resulting chromatograms.

The Office is therefore placing an unwarranted and improper burden upon the Applicants. The claims **do not** require that the method detect "all possible classes of novel compounds" produced by the transformed hosts. The claims **do not** require that the compounds detected be "novel." The claims do not require isolating the detected compounds. The claims require only that "a compound" produced by the transformed host cells as a result of transforming them to contain the isolated genomic DNA be detected. In other words, the claims require only the detection of a compound produced by the transformed host cell that is not produced by the unmodified host cell.

Moreover, and contrary to the assertion made at page 8 (first full paragraph) of the Office Action, the specification contains extensive directions on several different methods by which the compounds can be detected. In this regard, Applicants note that the key word in the phrase "undue experimentation" is "undue," and not "experimentation." See *In re Angstadt*, 190 USPQ 214, 219 (CCPA 1976). A truly large amount of experimentation is permissible if it is merely routine, or if the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. See also *In re Jackson*, 217 USPQ 804 (Bd. App. 1982). Thus, for example, chromatographic and spectrometric techniques are exceedingly well-known in the art and extensively used in industry. Any number of well-

known reference books are available to the skilled practitioner. Many of these techniques are automated and run robotically. The resulting spectra can be compared electronically. Even if the chosen detection technique is time-consuming and laborious, it is not "undue" as per the patent law if the detection technique is "routine."

In this regard, note that there are several prominent scientific journals limited entirely to, for example, spectrophotometric and chromatographic techniques. The person of ordinary skill in the art can easily access *Applied Spectroscopy Reviews*, published by Marcel Dekker, Inc. (New York, NY). See Exhibit B, attached hereto and incorporated herein. As noted by the publisher:

> *Applied Spectroscopy Reviews* provides the latest information on the principles, methods, and applications of all the diverse branches of spectroscopy. From X-ray, infrared, Raman, atomic absorption, and ESR to microwave, mass, NQR, NMR, and ICP, this international, single-source journal presents discussions that relate physical concepts to chemical applications for chemists, physicists, and other scientists using spectroscopic techniques.

Other easily-accessible journals along these lines include *The Journal of Applied Spectroscopy* (Kluwer Academic Publishers), *The Journal of Molecular Spectroscopy* (Academic Press/Elsevier), *The Journal of Analytical Atomic Spectroscopy* (RSC/Royal Society of Chemistry), etc. An equally large number of journals and reference works are devoted entirely to chromatographic detection techniques: *The Journal of Chromatography* (Elsevier), *The Journal of Liquid Chromatography* (Marcel Dekker), and the *Journal of Chromatographic Sciences* (Preston Publications), among many other works.

Moreover, Applicants submit that the specification contains a wealth of information, including extensive citations to the prior art, for various methods to detect compounds produced by the transformed host cells. In particular, the Office's attention is directed to pages 29-39 of the specification for an extensive discussion of various detection methods. Method specifically addressed for detecting new compound produced by the transformed host cells including classical fractionation techniques (such as those used by the Curies to detect polonium and radium), *e.g.*, chromatographic separation, or solvent fractionation. Also described are photometric techniques (*e.g.*, fluorescence or

phophorescence) for detecting analogs of known compounds. See page 30 and the top of page 31 of the specification.

Page 31 of the specification describes assaying the transformed cells by contacting a test sample from the transformed cells with a test cell. As noted in the first full paragraph on page 31:

> For instance, a test cell... is contacted with conditioned media (whole or fractionated) from a recombinant host cell, and the ability of the conditioned media to induce a biological or biochemical response [in] the [test] cell is assessed.

The passage goes on to state that the assay can be arranged to detect, for example, a phenotypic change in the test cell, such as a change in the transcriptional or translational rate or splicing pattern of a gene; the stability of a protein; the phosphorylation, prenylation, methylation, glycosylation, or other post-translational modification of a protein, nucleic acid, or lipid; the production of 2nd messengers, such as cAMP, inositol phosphates, and the like. The passage at page 31 explicitly indicates that such effects can be measure directly (such as by isolating and studying a particular component of the test cell) or indirectly such as by the expression of a reporter gene, or via cytotoxic or cytostatic activity imparted to the test cell.

Very specific assays that can be used to detect compounds are recited starting at the bottom of page 31 of the specification. Thus, the specification explicitly discusses detecting compound by measuring changes in GTPase activity of cAMP production between the unmodified host cells and the transformed host cells.

The paragraph spanning pages 31 and 32 discusses using the activity of phospholipase C, as a means to compare the unmodified host cells to the transformed host cells. The second full paragraph of page 32 describes using the activity of phospholipase C as a means to detect new compound produced by the transformed host cells. Assaying for changes to cellular phosphorylation is also discussed at page 32 of the specification. Here, the MAP kinase pathway is explicitly offered as an exemplary pathway that can be used as an assay.

Page 33 of the specification discuss using a substrate that, when converted to a particular product, will produce a detectable change in the optical characteristics of the test cell; *e.g.*, one of the substrate or its resulting product are chromogenically or

fluorgenically active – thus a new compound produced by the transformed cells that acts upon the substrate can be detected by measuring an increase or decrease in, for example, the fluorescent signal generated by the added substrate. Several suitable substrates are mentioned, along with five (5) prior art patents and one (1) prior art paper that describe these types of assays.

The first full paragraph of page 33 discusses detecting the compound by using enzymes or fluorescent probes whose activities are dependent upon the concentration of a second messenger compound (potentially produced by the transformed cells).

Pages 35-36 of the specification discuss using reporter genes and transcriptional regulatory elements to detect compounds produced by the transformed host cells that are not produced by the unmodified host cells.

Cell-free detection systems are discussed starting at the top of page 37 of the specification. Many of these assays measuring the formation of a protein complex as a primary screen to detect new compounds. As noted in the third full paragraph of page 37, the amount of target complex formed can be measured by labeling, by immunoassay, or by chromatographic detection.

Using an interactive trap assay to detect compounds produced by the transformed cells that are not produced by the unmodified cells is discussed at the bottom of page 37 of the specification. Citations to six (6) prior art references are supplied to provide ample guidance to the skilled practitioner.

Enzyme assays to detect compounds produced by the transformed host cells are discussed in detail at the middle page 38 of the specification.

A specific working example is provided at page 40, section (C) of the specification, namely, taking conditioned culture medium from the transformed cells and testing it for nematicidal activity against *C. elegans*, and then following up with insecticidal activity against a variety of insects.

In short, the specification contains a rather lengthy discussion of a host of different methods by which the compounds can be detected. None of these methods require any prior knowledge of any physical characteristic of the compound to be discovered. The claims do not require that the compound be identified, but only that its presence be detected.

Lastly, Applicants note that not everything necessary to practice the invention needs to be disclosed in the specification. See MPEP §2164.08. In fact, the MPEP and the relevant case law explicitly encourage applicants to omit from the specification that which is well known in the art. See *In re Buchner*, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). As noted above, the practitioner of ordinary skill has access to a huge volume of literature regarding chemical detection techniques, and the specification contains a rather lengthy description of a representative sampling of these techniques.

For these reasons, Applicants submit that the rejection of Claims 2-30, and 32 under §112, first paragraph (enablement) is improper. Withdrawal of the rejection is respectfully requested.

Rejection of Claims 2, 8-11, 13, and 15-24 Under 35 USC §102(e) Over Short et al. (U.S. Patent No. 6,057,103):

This rejection is respectfully traversed because the Short et al. patent fails to disclose cloning environmental DNA without an intervening step of isolating or culturing cells from the source. In short, throughout the Short et al. patent, the sample to be analyzed was treated to "enrich" the bacteria present in the sample. Only after the enrichment step do Short et al. extract the desired bacterial genomic DNA. What Short et al. refer to as "enrichment" is nothing more than applying a selective culture medium to the sample. In this regard, the Office's attention is directed to column 7, 2nd and 3rd full paragraphs of the Short et al. reference:

> The microorganisms from which the libraries may be prepared may be collected using a variety of techniques known in the art. Samples may also be collected using the methods detailed in the example provided below. Briefly, the example below provides a method of selective **in situ enrichment** of bacterial and archaeal species while at the same time inhibiting the proliferation of eukaryotic members of the population. In situ enrichments can to increase the likelihood of recovering rare species and previously uncultivated members of a microbial population. If one desires to obtain bacterial and archaeal species, nucleic acids from eukaryotes in an environmental sample can seriously complicate DNA library construction and decrease the number of desired bacterial species by grazing. The method described below employs selective agents, such as antifungal agents, to inhibit the growth of eukaryotic species.
>
> In situ enrichment is achieved by using a microbial containment device composed of growth substrates and nutritional amendments with the intent to lure,

> selectively, members of the surrounding environmental matrix. Choice of substrates (carbon sources) and nutritional amendments (i.e., nitrogen, phosphorous, etc.) is dependent upon the members of the community for which one desires to enrich. Selective agents against eukaryotic members are also added to the trap. Again, the exact composition depends upon which members of the community one desires to enrich and which members of the community one desires to inhibit. Some of the enrichment "media" which may be useful in pulling out particular members of the community is described in the example provided herein.

As this passage makes clear, Short et al. explicitly teach that "growth substrates" and "nutritional amendments" must be added to the sample in order to encourage growth of the desired cell types (bacteria in this instance), while simultaneously discouraging the growth of unwanted cell types (eukaryotes and fungi). This is the very definition of selective culturing. See Exhibit C, which is a definition of "selective medium" taken from the website of the Biochemistry Department of Northwestern University:

> **selective medium**: a culture medium that is enriched with a particular substance to allow the growth of particular strains of organisms.

The definition of "selective medium" tracks Short et al's definition of "enrichment" exactly. Moreover, Short et al's Example 1, starting at column 20, line 15, uses this "selective enrichment" of the sample prior to extracting DNA from the sample. In particular, note that Short et al. **explicitly state**, without equivocation, that the enrichment takes place prior to isolating the DNA from the sample. See Short et al., column 20, lines 18-21: "[The] sample to be utilized **for downstream nucleic acid isolation** for library generation may be collected according to the following example." (Emphasis added.) In other words, according to Short et al. enrichment (*i.e.* culturing) of the sample takes place first, **and then** the DNA is isolated from the sample ("downstream").

In the same vein, Example 3 of Short et al. explicitly describes **isolating** and **incubating** the cells from the sample prior to extracting DNA from the cells. See Short et al., column 23, line 48, to column 24, line 6:

> Agarose plugs containing concentrated picoplankton cells were prepared from samples collected on an oceanographic cruise from Newport, Oreg. to Honolulu, HI. Seawater (30 liters) was collected in Niskin bottles, screened through 10 μm Nitex, and concentrated by hollow fiber filtration (Amicon DC10) through 30,000

MW cutoff polysulfone filters. The concentrated bacterioplankton cells were collected on a 0.22 μm, 47 mm Durapore filter, and resuspended in 1 ml of 2x STE buffer (1M NaCl, 0.1M EDTA, 10 mM Tris, pH 8.0) to a final density of approximately 1.times.10.sup.10 cells per ml. The cell suspension was mixed with one volume of 1% molten Seaplaque LMP agarose (FMC) cooled to 40.degree. C., and then immediately drawn into a 1 ml syringe. The syringe was sealed with parafilm and placed on ice for 10 min. The cell-containing agarose plug was extruded into 10 ml of Lysis Buffer (10 mM Tris pH 8.0, 50 mM NaCl, 0.1M EDTA, 1% Sarkosyl, 0.2% sodium deoxycholate, a mg/ml lysozyme) and incubated at 37.degree. C. for one hour. The agarose plug was then transferred to 40 mls of ESP Buffer (1% Sarcosyl, 1 mg/ml proteinase-K, in 0.5M EDTA), and incubated at 55.degree. C. for 16 hours. The solution was decanted and replaced with fresh ESP Buffer, and incubated at 55.degree. C. for an additional hour. The agarose plugs were then placed in 50 mM EDTA and stored at 4.degree. C. shipboard for the duration of the oceanographic cruise.

As is clear from the cited passage, the cells contained in the water samples Short et al. used in their Example 3 were extensively concentrated and isolated prior to the cells being lysed and the DNA extracted.

The distinction between the claimed invention and Short et al's invention is that Short's manipulation of the environmental samples necessarily skews the DNA that is ultimately isolated from the sample. By definition, enriching a sample for particular types of bacteria (as Short et al. do in their Example 1) necessarily results in other organisms being excluded from the sample. Likewise, by isolating the picoplankton from the seawater in Example 3, Short et al. necessarily exclude other types of marine organisms from the sample.

As a result, the genomic DNA that Short et al. isolate from their samples **is not** representative of the diversity of open reading frame sequences present in the original sample. Inescapably and inexorable, Short et al. are purposefully throwing away significant portions of the diverse life forms present in the samples they actually tested and in their exemplary protocol. Rather than extracting the DNA directly from the sample, Short et al. extensively manipulate the samples, either by selectively culturing the samples (to enrich certain populations of the microorganisms found in the sample), or by isolating the cells (as in the case of Short et al.'s Example 3, at column 23).

In contrast, in the present invention, the genomic DNA is isolated directly from the source, without isolating the cells contained therein and without culturing the cells.

This yields genomic DNA that is representative of the diverse life forms to be found within the sample. The present invention proceeds in the absence of an enrichment step that takes place prior to DNA isolation. In the claimed invention, the DNA is isolated first, without culturing the sample, without enriching the sample, and without isolating the cells present in the sample.

Because Short et al. do not disclose such a method, Applicants respectfully submit that the rejection of Claims 2, 8-11, 13, and 15-24 Under 35 USC §102(e) Over Short et al. has been overcome. Withdrawal of the rejection is respectfully requested.

Rejection of Claims 2-8, 10, 13-22, 24-29, 30, and 41-45 Under 35 USC §102(e) Over Thompson et al. (U.S. Patent No. 5,824,485):

As applied to 42-45, this rejection has been rendered moot by cancellation of the claims.

Regarding new Claims 46-48, Applicants note that Claim 46 explicitly requires that the supernatant be extracted with chloroform "in the absence of phenol." This has been found by the present inventors to be more gentle than using phenol-chloroform for extraction and yields genomic DNA that is better preserved as compared to the DNA extracted using phenol-chloroform. The Thompson et al. patent relies entirely on phenol/chloroform extraction. See Thompson et al., column 28, lines 7-9; column 41, lines 35-39; and column 43, lines 40-45.

Claims 46-48 also require a size-fractionation step that takes place prior to the DNA being enzymatically treated. As noted in the Rule 132 Declarations in the now-issued parent application, copies of which are submitted herewith as Exhibits D, E, and F, subjecting the DNA to a size-fractionation purification step, such as pulsed-field gel electrophoresis, prior to enzymatic manipulation of the DNA for insertion into the vector, improves the quality of the resultant vector. See paragraph 5 of each Declaration. (Note that the Declarations attached are exact duplicates of the Declarations filed in the parent application, with the exception that the serial number on the front of each Declaration has been changed to that of the present application to ensure that the Declaration are properly matched with the present file and not the parent file.)

As applied to the remaining claims, this rejection is believed to have been overcome by appropriate amendment to the claims.

In particular, the Thompson et al. patent is limited to libaries constructed using the cosmid "SuperCos 1." See Thompson et al., column 21, first full paragraph:

> For instance, a preferred and exemplary expression vector-host organism combination is the cosmid, SuperCos 1 and the Esherichia coli strain, XL1-Blue MR, both of which are commercially available from Stratagene (La Jolla, Calif.). The vector accepts through a BamHI cloning site DNA inserts **ranging from 30-42 kbp** in size, and carries a neomycin resistance marker (neoR) and an SV40 promoter that is used for expression in mammalian cell. The vector also contains an ampicillin resistance gene for selection in prokaryotic cells. The E. coli host organism is deficient in certain restriction systems (hsdR, mcrA, mcrCB and mrr), is endonuclease-deficient (endA1), and recombination deficient (recA). The host organism cannot cleave inserted DNA carrying cytosine and/or adenine methylation, which is often present in eukaryotic DNA and cDNA synthesized using methyl-dNTP analogs. (Emphasis added.)

In short, the Thompson et al. patent is wholly silent with regard to fabricating libraries containing inserts at least 50 kb in length and libraries wherein the average insert length is at least 50 kb.

The Office's attention is also directed to paragraph 8 of the Declarations attached hereto as Exhibits D, E, and F. As noted in paragraph 8 of the Declarations, while Thompson et al. describe isolating DNA from soil or mixed environment samples, the inventors are of the scientific opinion that the steps described by Thompson et al. would not result in libraries have large inserts (*i.e.*, 50 kb and larger).

Applicants submit that the amendment to the claims and the Declarations attached hereto combine to overcome the §102(e) rejection in view of the Thompson et al. patent. Withdrawal of the rejection is respectfully requested.

Rejection of Claims 2, 10, 11, 12, and 14 Under 35 USC §103(a) Over Short et al. in View of Fuerst et al. and Stein et al.:

This rejection is believed to have been overcome by appropriate amendment to the claims. As noted above, the Short et al. patent fails to disclose cloning environmental DNA without an intervening step of isolating or culturing cells from the source. Throughout the Short et al. patent, the sample to be analyzed is treated to "enrich" the

bacteria present in the sample. In the Short et al. process, only after the enrichment step is the bacterial genomic DNA isolated. In effect, Short et al. use a selective culture medium to culture bacteria present in the sample and to exclude non-bacterial species. See column 7, 2nd and 3rd full paragraphs of the Short et al. reference, cited *supra*.

Combining Short et al. with Fuerst et al. and Stein et al. does not cure the shortcomings of the primary reference because the combination of all three reference still fails to disclose or suggest isolating the DNA in the absence of either isolating the cells from the sample or culturing the cells as disclosed in the Short et al. patent.

The Fuerst et al. document is simply an introduction to the classification of microorganisms. Applicants certainly **are not** asserting that they discovered the difference between Eubacteria and Archaebacteria. The Fuerst et al. document teaches nothing further. It is nothing more than a very brief and concise introduction to the classification of microorganisms generally and prokaryotes particularly.

The Stein et al. paper is limited to the characterization of a 40-kb genome fragment from a planktonic marine organism classified as an Archaeon. The cloning, however, was done in fosmids, an *E. coli* F-factor based cosmid. But, as noted by Thompson et al. (in the passage at column 21, first full paragraph, and cited in the immediately preceding section), a cosmid will not package a DNA insert larger than 42 kb. The present claims, however, require that at least some of the clones have an insert size of at least 50 kb.

Thus, when all three references are combined, the result is Short et al.'s approach, where the sample would be selectively cultured or the cells would be isolated from the remainder of the sample. The cells would then be confirmed as Archaebacteria as per the teaching of Fuerst et al. The DNA would then be isolated as per Short et al. or Stein et al. The resulting DNA would necessarily be in fragments far smaller than 50 kb in order to be inserted in the fosmid vector dictated by Stein et al. As a result, none of the clones resulting from this combination of references would have an insert size approaching 50 kb, as required by the positive language of Claim 2.

For these reasons, Applicants respectfully submit that the rejection under §103(a) over the combination of Short et al, Fuerst et al., and Stein et al. has been overcome. Withdrawal of the same is respectfully requested.

CONCLUSION

Applicants submit that the application is now in condition for allowance. Early notification of such action is earnestly solicited.

Respectfully submitted,

Joseph T. Leone, Reg. No. 37,170
DEWITT ROSS & STEVENS S.C.
8000 Excelsior Drive, Suite 401
Madison, Wisconsin 53717-1914
Telephone: (608) 831-2100
Facsimile: (608) 831-2106

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lateral science

THE DREAM BECOMES A REALITY
THE DISCOVERY OF RADIUM by Marie Curie

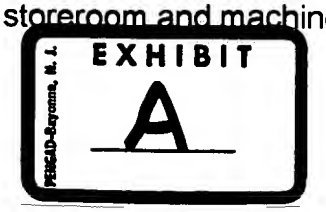
I HAVE already said that in 1897 Pierre Curie was occupied with an investigation on the growth of crystals. I myself had finished, by the beginning of vacation, a study of the magnetization of tempered steels which had resulted in our getting a small subvention from the Society for the Encouragement of National Industry. Our daughter Irène was born in September, and as soon as I was well again, I resumed my work in the laboratory with the intention of preparing a doctor's thesis.

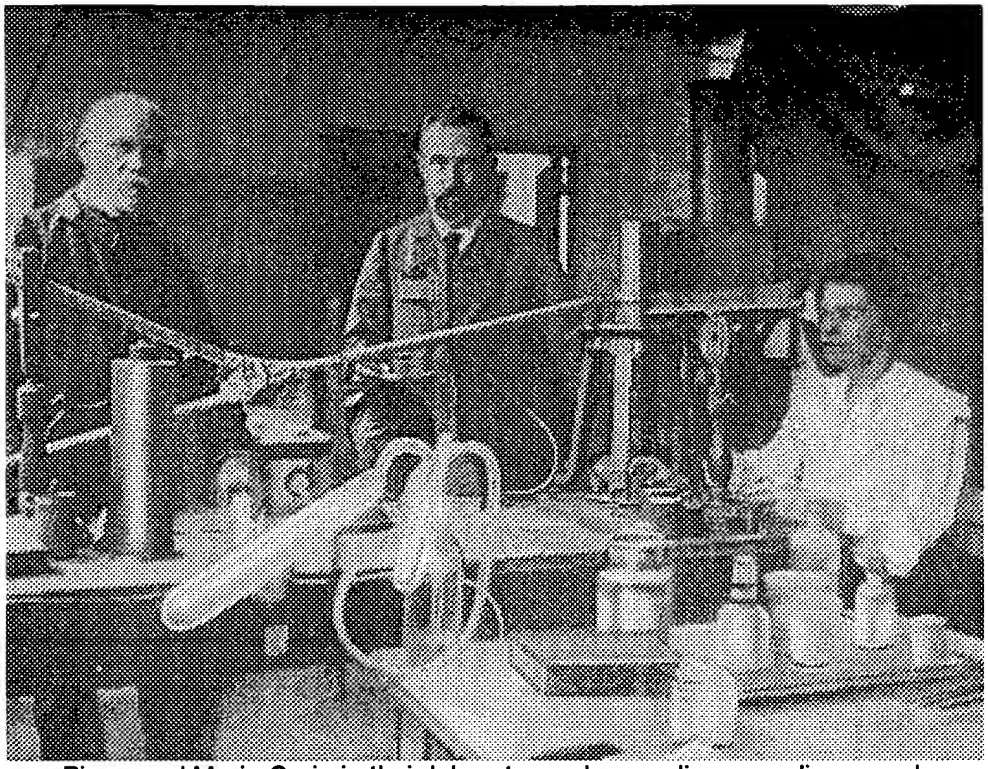
Our attention was caught by a curious phenomenon discovered in 1896 by Henri Becquerel. The discovery of the X-ray by Roentgen had excited the imagination, and many physicians were trying to discover if similar rays were not emitted by fluorescent bodies under the action of light. With this question in mind Henri Becquerel was studying uranium salts, and, as sometimes occurs, came upon a, phenomenon different from that he was looking for: the spontaneous emission by uranium salts of rays of a peculiar character. This was the discovery of radioactivity.

The particular phenomenon discovered by Becquerel was as follows: uranium compound placed upon a photographic plate covered with black paper produces on that plate an impression analogous to that which light would make. The impression is due to uranium rays that traverse the paper. These same rays can, like X-rays, discharge an electroscope, by making the air which surrounds it a conductor.

Henri Becquerel assured himself that these properties do not depend on a preliminary isolation, and that they persist when the uranium compound is kept in darkness during several months. The next step was to ask whence came this energy, of minute quantity, it is true, but constantly given off by uranium compounds under the form of radiations.

The study of this phenomenon seemed to us very attractive and all the more so because the question was entirely new and nothing yet had been written upon it. I decided to undertake an investigation of it. It was necessary to find a place in which to conduct the experiments. My husband obtained from the director of the School the authorization to use a glassed-in study on the ground floor which was then being used as a storeroom and machine shop.





Pierre and Marie Curie in their laboratory, where radium was discovered.

In order to go beyond the results reached by Becquerel, it was necessary to employ a precise quantitative method. The phenomenon that best lent itself to measurement was the conductibility produced in the air by uranium rays. This phenomenon, which is called ionization, is produced also by X-rays and investigation of it in connection with them had made known its principal characteristics.

For measuring the very feeble currents that one can make pass through air ionized by uranium rays, I had at my disposition an excellent method developed and applied by Pierre and Jacques Curie. This method consists in counterbalancing on a sensitive electrometer the quantity of electricity carried by the current with that which a piezo-electric quartz can furnish. The installation therefore required a Curie electrometer, a piezo-electric quartz, and a chamber of ionization, which last was formed by a plate condenser whose higher plate was joined to the electrometer, while the lower plate, charged with a known potential, was covered with a thin layer of the substance to be examined.

Needless to say, the place for such an electrometric installation was hardly the crowded and damp little room in which I had to set it up. My experiments proved that the radiation of uranium compounds can be measured with precision under determined conditions, and that this radiation is an atomic property of the element of uranium. Its intensity is proportional to the quantity of uranium contained in the compound, and depends neither on conditions of chemical combination, nor on external circumstances, such as light or temperature.

I undertook next to discover if there were other elements possessing the same property, and with this aim I examined all the elements then known, either in their pure state or in compounds. I found that among these bodies, thorium compounds are the only ones which emit rays similar to those of uranium. The radiation of thorium has an intensity of the same order as that of uranium, and is, as in the case of uranium, an atomic property of the element.

It was necessary at this point to find a new term to define this new property of matter manifested by the elements of uranium and thorium. I proposed the word radioactivity which has since become generally adopted; the radioactive elements have been called radio elements.

During the course of my research, I had had occasion to examine not only simple compounds, salts and oxides, but also a great number of minerals. Certain ones proved radioactive; these were those containing uranium and thorium; but their radioactivity seemed abnormal, for it was much greater than the amount I had found in uranium and thorium had led me to expect.

This abnormality greatly surprised us. When I had assured myself that it was not due to an error in the experiment, it became necessary to find an explanation. I then made the hypothesis that the ores uranium and thorium contain in small quantity a substance much more strongly radioactive than either uranium or thorium. This substance could not be one of the known elements, because these had already been examined; it must, therefore, be a new chemical element.

I had a passionate desire to verify this hypothesis as rapidly as possible. And Pierre Curie, keenly interested in the question, abandoned his work on crystals (provisionally, he thought) to join me in the search for this unknown substance.

We chose, for our work, the ore pitchblende, a uranium ore, which in its pure state is about four times more active than oxide of uranium. Since the composition of this ore was known through very careful chemical analysis, we could expect to find, at a maximum, 1 per cent of new substance. The result of our experiment proved that there were in reality new radioactive elements in pitchblende, but that their proportion did not reach even a millionth per cent!

The method we employed is a new method in chemical research based on radioactivity. It consists in inducing separation by the ordinary means of chemical analysis, and of measuring, under suitable conditions, the radioactivity of all the separate products. By this means one can note the chemical character of the radioactive element sought for, for it will become concentrated in those products which will become more and more radioactive as the separation progresses. We soon recognized that the radioactivity was concentrated principally in two different chemical fractions, and we became able to recognize in pitchblende the presence of at least two new radioactive elements: polonium and radium. We announced the existence of polonium in July, 1898, and of radium in December of the same year.

In spite of this relatively rapid progress, our work was far from finished. In our opinion, there could be no doubt of the existence of these new elements, but to make chemists admit their existence, it was necessary to isolate them. Now, in our most strongly radioactive products (several hundred times more active than uranium), the polonium and radium were present only as traces. The polonium occurred associated with bismuth extracted from pitchblende, and radium accompanied the barium extracted from the same mineral. We already knew by what methods we might hope to separate polonium from bismuth and radium from barium; but to accomplish such a separation we had to have at our disposition much larger quantities of the primary ore than we had. It was during this period of our research that we were extremely handicapped by inadequate conditions, by the lack of a proper place to work in, by the lack of money and of personnel.

Pitchblende was an expensive mineral, and we could not afford to buy a sufficient quantity. At

that time the principal source of this mineral was at St. Joachimsthal (Bohemia) where there was a mine which the Austrian government worked for the extraction of uranium. We believed that we would find all the radium and a part of the polonium in the residues of this mine, residues which had so far not been used at all. Thanks to the influence of the Academy of Sciences of Vienna, we secured several tons of these residues at an advantageous price, and we used it as our primary material. In the beginning we had to draw on our private resources to pay the costs of our experiment; later we were given a few subventions and some help from outside sources.

The question of quarters was particularly serious; we did not know where we could conduct our chemical treatments. We had been obliged to start them in an abandoned storeroom across a court from the workroom where we had our electrometric installation. This was a wooden shed with a bituminous floor and a glass roof which did not keep the rain out, and without any interior arrangements. The only objects it contained were some worn pine tables, a cast-iron stove, which worked badly, and the blackboard which Pierre Curie loved to use. There were no hoods to carry away the poisonous gases thrown off in our chemical treatments, so that it was necessary to carry them on outside in the court, but when the weather was unfavorable we went on with them inside, leaving the windows open.

A view of the extraction of radium in the old shed where the first radium was obtained

In this makeshift laboratory we worked practically unaided during two years, occupying ourselves as much with chemical research as with the study of the radiation of the increasingly active products we were obtaining. Then it became necessary for us to divide our work. Pierre Curie continued the investigations on the properties of radium, while I went ahead with the chemical experiments which had as their objective the preparation of pure radium salts. I had to work with as much as twenty kilogrammes of material at a time, so that the hangar was filled

with great vessels full of precipitates and of liquids. It was exhausting work to move the containers about, to transfer the liquids, and to stir for hours at a time, with an iron bar, the boiling material in the cast-iron basin. I extracted from the mineral the radium-bearing barium and this, in the state of chloride, I submitted to a fractional crystallization.

The radium accumulated in the least soluble parts, and I believed that this process must lead to the separation of the chloride of radium. The very delicate operations of the last crystallizations were exceedingly difficult to carry out in that laboratory, where it was impossible to find protection from the iron and coal dust. At the end of a year, results indicated clearly that it would be easier to separate radium than polonium; that is why we concentrated our efforts in this direction. We examined the radium salts we obtained with the aim of discovering their powers and we loaned samples of the salts to several scientists, in particular to Henri Becquerel.

During the years 1899 and 1900, Pierre Curie published with me a memoir on the discovery of the induced radioactivity produced by radium. We published another paper on the effects of the rays: the luminous effects, the chemical effects, etc.; and still another on the electric charge carried by certain of the rays. And, finally, we made a general report on the new radioactive substances and their radiations, for the Congress of Physics which met in Paris in 1900. My husband published, besides, a study of the action of a magnetic field on radium rays.

Pierre Curie with the quartz piezo-electroscope he invented, by which rays of radium are measured

The main result of our investigations and of those of other scientists during these years, was to make known the nature of the rays emitted by radium, and to prove that they belonged to three different categories. Radium emits a stream of active corpuscles moving with great speed.

Certain of them carry a positive charge and form the Alpha rays; others, much smaller, carry a negative charge and form Beta rays. The movements of these two groups are influenced by a magnet. A third group is constituted by the rays that are insensible to the action of a magnet, and that, we know to-day, are a radiation similar to light and to X-rays.

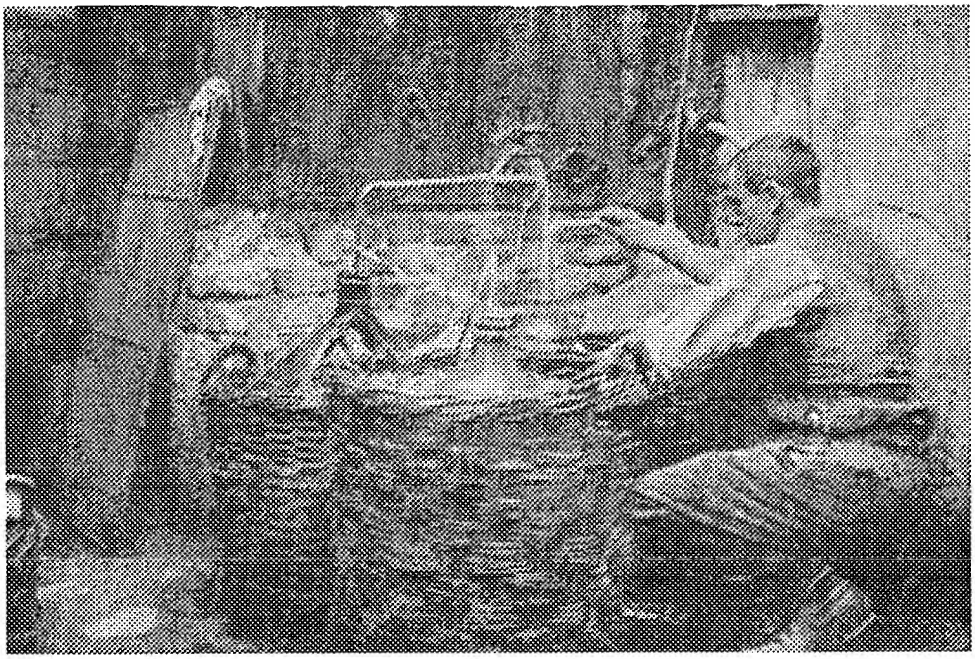
We had an especial joy in observing that our products containing concentrated radium were all spontaneously luminous. My husband who had hoped to see them show beautiful colorations had to agree that this other unhoped-for characteristic gave him even a greater satisfaction than that he had aspired to.

The Congress of 1900 offered us an opportunity to make known, at closer range, to foreign scientists, our new radioactive bodies. This was one of the points on which the interest of this Congress chiefly centered.

We were at this time entirely absorbed in the new field that opened before us, thanks to the discovery so little expected. And we were very happy in spite of the difficult conditions under which we worked. We passed our days at the laboratory, often eating a simple student's lunch there. A great tranquillity reigned in our poor, shabby hangar; occasionally, while observing an operation, we would walk up and down talking of our work, present and future. When we were cold, a cup of hot tea, drunk beside the stove, cheered us. We lived in a preoccupation as complete as that of a dream.

Sometimes we returned in the evening after dinner for another survey of our domain. Our precious products, for which we had no shelter, were arranged on tables and boards; from all sides we could see their slightly luminous silhouettes, and these gleamings, which seemed suspended in the darkness, stirred us with ever new emotion and enchantment.

Actually, the employees of the School owed Pierre Curie no service. But nevertheless the laboratory helper whom he had had to aid him when he was laboratory chief had always continued to help him as much as he could in the time at his disposal. This good man, whose name was Petit, felt a real affection and solicitude for us, and many things were made easier because of his good will and the interest be took in our success.



A view of the extraction of radium in the old shed where the first radium was obtained

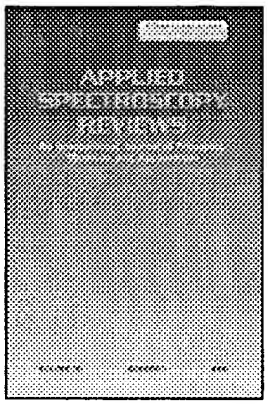
We had begun our research in radioactivity quite alone, but because of the magnitude of the undertaking, we were more and more convinced of the utility of inviting collaboration. Already in 1898, one of the laboratory chiefs of the School, G. Bemont, had given us temporary aid. And toward 1900 Pierre Curie associated with him a young chemist, André Debierne, preparator under Friedel, who held him in high esteem. André Debierne gladly accepted Pierre Curie's proposal that he occupy himself with the investigation of radioactivity; and he undertook, in particular, the search for a new radio element, which we suspected existed in the iron group and in rare earths. He discovered the element actinium. Even though he carried on his work in the laboratory of physical chemistry at the Sorbonne, directed by Jean Perrin, he frequently came to visit us in our storeroom, and was soon an intimate friend of ours, and of Doctor Curie and the children.

About this same time, George Sagnac, a young physicist engaged in the study of X-rays, often came to discuss with my husband the analogies one could expect to find between these rays, and their secondary rays, and the radiations of radioactive bodies. They worked together on the investigation of the electric charge carried by the secondary rays.

Besides our collaborators we saw very few persons in the laboratory; however, from time to time some physicist or chemist came to see our experiments, or to ask Pierre Curie for advice or information; for his authority in several branches of physics was very well recognized. And then there were discussions before the blackboard, -- discussions which are pleasantly remembered to-day, because they stimulated an interest in science and an ardor for work without interrupting any course of reflection, and without troubling that atmosphere of peace and contemplation which is the true atmosphere of the laboratory.

This article from chapter five - **Pierre Curie** by Marie Curie 1923

lateral science home page



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Edited by: J. Sneddon [1]

[1] *McNeese State University, Lake Charles, Louisiana, U.S.A.*

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EXHIBIT B

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selective medium

Definition:

A culture medium that is enriched with a particular substance to allow the growth of particular strains of organisms.

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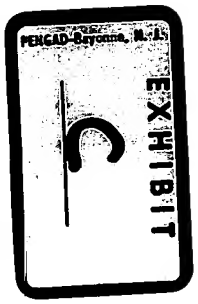

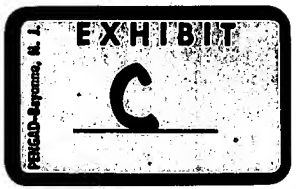

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Handelsman et al.	Group Art Unit: 1636
Serial No.: 09/877,406	Examiner: Garvey, Tara L.
Filed: June 8, 2001	
For: *Microorganism Genomics, Compositions and Methods Related Thereto*	Attorney Docket No.: 09820.365 (Formerly: AVI-001.03)

Assistant Commissioner for Patents
Washington, D.C. 20231

Declaration Under 37 C.F.R. § 1.132 by Jo Handelsman

1. I, Jo Handelsman, of Madison, Wisconsin, hereby declare as follows:

2. I am Professor of plant pathology in the Department of Plant Pathology at the University of Wisconsin-Madison since 1995. I was an Associate Professor from 1991 to 1995 and an Assistant Professor from 1985 to 1991 at the same Department. Prior to holding this position, I was a postdoctoral fellow in the Department of Plant Pathology at the University of Wisconsin-Madison (1984-1985) and a graduate student in the Molecular Biology Graduate Program at the University of Wisconsin (1979-1984).

3. I am a co-inventor in the above-referenced patent application.

4. I have reviewed the present application (herein, the "Specification"), the pending claims, and the Office Actions mailed July 5, 2000; October 15, 1999; and January 21, 1999 (herein, the "Office Actions"). I understand that the Examiner has rejected the pending claims 1, 3-5, 8-16, 19-24, 31, 34, 35, and 40, on the grounds that they are anticipated by Short et al. (WO 97/04077); Short et al. (U.S. patent 5,958,672; the two Short et al. references are together referred to herein as "Short et al."); and Thompson et al. (U.S. patent 5,824,485). I have reviewed the Short et al. and Thompson et al. references, and respectfully disagree with the rejections.

5. Myself and the other two co-inventors devised a method for preparing a library of host cells containing clones of a vector comprising genomic DNA from a source containing microorganisms, e.g., soil, wherein the genomic DNA in at least some of the clones is at least 50 kb. The method enables preparation of libraries of host cells containing large genomic fragments from microorganisms present in a source without requiring the isolation of the microorganisms from the source prior to extraction of the genomic DNA

from the microorganisms. Thus, the method is efficient for preparing libraries of genomic DNA from sources which contain high amounts of impurities, e.g., soil, or from which it is difficult to isolate and/or culture the microorganisms. Generally, the method includes mixing the source with an extraction buffer to isolate the DNA; inserting the DNA into a vector to form a clone; and transforming host cells with the clones. We have unexpectedly found that, in order to obtain sufficient quantities of large genomic fragments of sufficiently good quality from a source containing microorganisms without having to first isolate the microorganims from the source, to construct libraries of host cells containing large inserts of the genomic DNA, it is necessary to mix the source with an extraction buffer and to subject the resulting mixture to freeze-thawing. Without wanting to be limited to a mechanisms of action of this step, we believe that this step may improve the quantity and/or quality of the DNA recovered by breaking up of microorganisms which may not have not been broken up by the extraction buffer. We believe that other gentle physical means for breaking up of microorganisms may also be used instead of freeze-thawing. We have also unexpectedly found that subjecting the genomic DNA isolated to a purification step, such as an electrophoresis step, e.g., agarose or pulse field electrophoresis, prior to enzymatic manipulation, e.g., restriction digestion, of the DNA for insertion into the vector, increases the quality of the library, i.e., the number of clones with large inserts and the size of the inserts, probably by improving the quantity and/or quality of the genomic DNA recovered. For example, we have subjected the DNA isolated from the source to preparative gel electrophoresis, prior to digestion with a restriction enzyme (see Example 2, at page 58, of the specification).

6. Using the method described in Example 2 of the specification including the two steps described in the previous paragraph, we were able to obtain libraries of host cells, at least some of which contain an insert of at least 50 kb. A first library is described in Example 2, at page 58, of the specification. This library was prepared from a sample of soil and contains inserts having a size ranging from 13 to 60 kb. As stated in the Example, such large fragments were obtained even when using methods that shear DNA during isolation, and we believe that inserts of over 100 kb could have been obtained if gentler methods had been used. Following the same method as that described in Example 2 and as that claimed, we have also obtained another library containing inserts of up to at least 80 kb, after the filing date of the application.

7. Short et al. do not teach or suggest a method for preparing a library from a source containing microorganisms, such as soil, in which microorganisms are not isolated from the source prior to isolation of their genomic DNA. Short et al. do not describe any protocol for isolating DNA from a source containing microorganisms without prior isolation of the microoganisms from the source. The only protocols described by Short et al. involved first isolating and concentrating the microorganisms (see Example 3). In

addition, in that example, the method utilizes agarose plugs, i.e., embedding of the concentrated bacteria in an agarose plug. Unlike Short et al., our method does not involve the use of an agarose plug. We have found that it is not possible to use the agarose plug method for isolating genomic DNA from soil to construct the claimed libraries, probably due to the presence of large amounts of impurities. In addition, Short et al. does not teach mixing the source with an extraction buffer and subjecting the resulting mixture to freeze-thawing. Thus, Short et al. does not anticipate the claimed invention. In addition, since the methods described in Short et al. are so different from our methods, and that it was unexpected that the additional two steps could lead to such a significant improvement in the quality of the library, it would not have been obvious to a person of skill in the art to modify the teachings of Short et al. in such a way as to obtain the claimed invention.

8. Thompson et al. fail to teach or suggest a method for preparing a library of clones of a vector comprising genomic DNA from a source containing microorganisms, wherein the microorganisms are not first isolated from the source prior to isolation of the genomic DNA from the source, and wherein the genomic DNA in at least some of the clones is at least 50 kb, or at least 20 kb in the case of soil DNA. All of the examples described in Thompson et al. include extracting DNA from isolated and cultured microoganisms. Although Thompson et al. describe in the specification a method for isolating DNA from soil or mixed environmental samples (section 5.3.6. at columns 41 and 42), we believe that this method alone would not allow isolation of sufficient quantities of large size DNA fragments to obtain libraries of host cells having large inserts. As further described above, we have unexpectedly found that certain steps must be included in a DNA extraction protocol to obtain sufficient DNA of large size to obtain the claimed libraries. Thompson et al. do not teach or suggest these steps. In addition, since it was unexpected that the additional two steps could lead to such a significant improvement in the quality of the library, it would not have been obvious to a person of skill in the art to modify the teachings of Thompson et al. in such a way as to obtain the claimed invention.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Jo Handelsman

Dated: January 18, 01

Signature: Jo Handelsman

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Handelsman et al.	Group Art Unit: 1636
Serial No.: 09/877,406	Examiner: Garvey, Tara L.
Filed: June 8, 2001	
For: *Microorganism Genomics, Compositions and Methods Related Thereto*	Attorney Docket No.: 09820.365 (Formerly: AVI-001.03)

Assistant Commissioner for Patents
Washington, D.C. 20231

Declaration Under 37 C.F.R. § 1.132 by Michelle R. Rondon

1. I, Michelle R. Rondon, of Columbus, Ohio, hereby declare as follows:

2. I am an Assistant Professor of Microbiology at the Department of Microbiology at the Ohio State University, Columbus, Ohio since January 2000. Prior to holding this position, I was a postdoctoral fellow since July 1996 in the laboratories of Profs R. Goodman and J. Handelsman in the department of Plant Pathology at the University of Wisconsin-Madison. I have obtained my Ph.D. at the University of Wisconsin-Madison in 1995.

3. I am a co-inventor in the above-referenced patent application.

4. I have reviewed the present application (herein, the "Specification"), the pending claims, and the Office Actions mailed July 5, 2000; October 15, 1999; and January 21, 1999 (herein, the "Office Actions"). I understand that the Examiner has rejected the pending claims 1, 3-5, 8-16, 19-24, 31, 34, 35, and 40, on the grounds that they are anticipated by Short et al. (WO 97/04077); Short et al. (U.S. patent 5,958,672; the two Short et al. references are together referred to herein as "Short et al."); and Thompson et al. (U.S. patent 5,824,485). I have reviewed the Short et al. and Thompson et al. references, and respectfully disagree with the rejections.

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concentrated bacteria in an agarose plug. Unlike Short et al., our method does not involve the use of an agarose plug. We have found that it is not possible to use the agarose plug method for isolating genomic DNA from soil to construct the claimed libraries, probably due to the presence of large amounts of impurities. In addition, Short et al. does not teach mixing the source with an extraction buffer and subjecting the resulting mixture to freeze-thawing. Thus, Short et al. does not anticipate the claimed invention. In addition, since the methods described in Short et al. are so different from our methods, and that it was unexpected that the additional two steps could lead to such a significant improvement in the quality of the library, it would not have been obvious to a person of skill in the art to modify the teachings of Short et al. in such a way as to obtain the claimed invention.

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9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Michelle R. Rondon

Dated: January 18, 2001

Signature: Michelle R. Rondon

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Handelsman et al. Serial No.: 09/877,406 Filed: June 8, 2001 For: *Microorganism Genomics, Compositions and Methods Related Thereto*	Group Art Unit: 1636 Examiner: Garvey, Tara L. Attorney Docket No.: 09820.365 (Formerly: AVI-001.03)

Assistant Commissioner for Patents
Washington, D.C. 20231

Declaration Under 37 C.F.R. § 1.132 by Robert M. Goodman

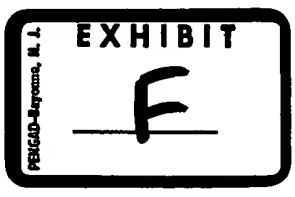
1. I, Robert M. Goodman, of Madison, Wisconsin, hereby declare as follows:

2. I am a Professor of plant pathology in the Department of Plant Pathology at the University of Wisconsin—Madison since September 1991. Prior to holding this position I was Executive Vice-President for Research and Development at Calgene, Inc. (1982-1990), and assistant, associate, and full professor of plant pathology at the University of Illinois-Urbana (1974-1982).

3. I am a co-inventor in the above-referenced patent application.

4. I have reviewed the present application (herein, the "Specification"), the pending claims, and the Office Actions mailed July 5, 2000; October 15, 1999; and January 21, 1999 (herein, the "Office Actions"). I understand that the Examiner has rejected the pending claims 1, 3-5, 8-16, 19-24, 31, 34, 35, and 40, on the grounds that they are anticipated by Short et al. (WO 97/04077); Short et al. (U.S. patent 5,958,672; the two Short et al. references are together referred to herein as "Short et al."); and Thompson et al. (U.S. patent 5,824,485). I have reviewed the Short et al. and Thompson et al. references, and respectfully disagree with the rejections.

5. Myself and the other two co-inventors devised a method for preparing a library of host cells containing clones of a vector comprising genomic DNA from a source containing microorganisms, e.g., soil, wherein the genomic DNA in at least some of the clones is at least 50 kb. The method enables preparation of libraries of host cells containing large genomic fragments from microorganisms present in a source without requiring the isolation of the microorganisms from the source prior to extraction of the genomic DNA from the microorganisms. Thus, the method is efficient for preparing libraries of



genomic DNA from sources which contain high amounts of impurities, e.g., soil, or from which it is difficult to isolate and/or culture the microorganisms. Generally, the method includes mixing the source with an extraction buffer to isolate the DNA; inserting the DNA into a vector to form a clone; and transforming host cells with the clones. We have unexpectedly found that, in order to obtain sufficient quantities of large genomic fragments of sufficiently good quality from a source containing microorganisms without having to first isolate the microorganims from the source, to construct libraries of host cells containing large inserts of the genomic DNA, it is necessary to mix the source with an extraction buffer and to subject the resulting mixture to freeze-thawing. Without wanting to be limited to a mechanisms of action of this step, we believe that this step may improve the quantity and/or quality of the DNA recovered by breaking up of microorganisms which may not have not been broken up by the extraction buffer. We believe that other gentle physical means for breaking up of microorganisms may also be used instead of freeze-thawing. We have also unexpectedly found that subjecting the genomic DNA isolated to a purification step, such as an electrophoresis step, e.g., agarose or pulse field electrophoresis, prior to enzymatic manipulation, e.g., restriction digestion, of the DNA for insertion into the vector, increases the quality of the library, i.e., the number of clones with large inserts and the size of the inserts, probably by improving the quantity and/or quality of the genomic DNA recovered. For example, we have subjected the DNA isolated from the source to preparative gel electrophoresis, prior to digestion with a restriction enzyme (see Example 2, at page 58, of the specification).

6. Using the method described in Example 2 of the specification including the two steps described in the previous paragraph, we were able to obtain libraries of host cells, at least some of which contain an insert of at least 50 kb. A first library is described in Example 2, at page 58, of the specification. This library was prepared from a sample of soil and contains inserts having a size ranging from 13 to 60 kb. As stated in the Example, such large fragments were obtained even when using methods that shear DNA during isolation, and we believe that inserts of over 100 kb could have been obtained if gentler methods had been used. Following the same method as that described in Example 2 and as that claimed, we have also obtained another library containing inserts of up to at least 80 kb, after the filing date of the application.

7. Short et al. do not teach or suggest a method for preparing a library from a source containing microorganisms, such as soil, in which microorganisms are not isolated from the source prior to isolation of their genomic DNA. Short et al. do not describe any protocol for isolating DNA from a source containing microorganisms without prior isolation of the microoganisms from the source. The only protocols described by Short et al. involved first isolating and concentrating the microorganisms (see Example 3). In addition, in that example, the method utilizes agarose plugs, i.e., embedding of the

concentrated bacteria in an agarose plug. Unlike Short et al., our method does not involve the use of an agarose plug. We have found that it is not possible to use the agarose plug method for isolating genomic DNA from soil to construct the claimed libraries, probably due to the presence of large amounts of impurities. In addition, Short et al. does not teach mixing the source with an extraction buffer and subjecting the resulting mixture to freeze-thawing. Thus, Short et al. does not anticipate the claimed invention. In addition, since the methods described in Short et al. are so different from our methods, and that it was unexpected that the additional two steps could lead to such a significant improvement in the quality of the library, it would not have been obvious to a person of skill in the art to modify the teachings of Short et al. in such a way as to obtain the claimed invention.

8. Thompson et al. fail to teach or suggest a method for preparing a library of clones of a vector comprising genomic DNA from a source containing microorganisms, wherein the microorganisms are not first isolated from the source prior to isolation of the genomic DNA from the source, and wherein the genomic DNA in at least some of the clones is at least 50 kb, or at least 20 kb in the case of soil DNA. All of the examples described in Thompson et al. include extracting DNA from isolated and cultured microoganisms. Although Thompson et al. describe in the specification a method for isolating DNA from soil or mixed environmental samples (section 5.3.6. at columns 41 and 42), we believe that this method alone would not allow isolation of sufficient quantities of large size DNA fragments to obtain libraries of host cells having large inserts. As further described above, we have unexpectedly found that certain steps must be included in a DNA extraction protocol to obtain sufficient DNA of large size to obtain the claimed libraries. Thompson et al. do not teach or suggest these steps. In addition, since it was unexpected that the additional two steps could lead to such a significant improvement in the quality of the library, it would not have been obvious to a person of skill in the art to modify the teachings of Thompson et al. in such a way as to obtain the claimed invention.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Robert M. Goodman

Dated: 1/20/01

Signature: